

Rancidity inhibition study in frozen whole mackerel (*Scomber scombrus*) following flaxseed (*Linum usitatissimum*) extract treatment

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RESUMEN

Estudio de la inhibición de la rancidez en caballa entera congelada (*Scomber scombrus*) producida por tratamiento con extracto de lino (*Linum usitatissimum*).

Se ha estudiado el efecto del lino (*Linum usitatissimum*) en el desarrollo de rancidez en caballa entera congelada (*Scomber scombrus*). Para ello, caballas frescas fueron sumergidas en extractos acuosos de semillas de lino durante 60 min, congeladas a -80 °C durante 24 h y mantenidas congeladas (-20 °C) durante 12 meses. Se tomaron muestras del material inicial y tras 1, 3, 5, 7, 9 y 12 meses de congelación a -20 °C. Un experimento paralelo con pescado no tratado fue llevado a cabo en las mismas condiciones. El desarrollo de la rancidez fue medido por varios índices bioquímicos (ácidos grasos libres, peróxidos, dienos y trienos conjugados, productos secundarios de oxidación y actividad lipoxigenasa) y completado con análisis sensorial (piel, olor de la carne, consistencia y apariencia de la carne). Como resultado del tratamiento antioxidante, los peróxidos se degradaron más rápidamente ($p < 0.05$) después del mes 7, y por tanto, contenidos mayores ($p < 0.05$) de dienos y trienos conjugados pudieron ser detectados en el pescado tratado. El tratamiento antioxidante también condujo a un menor contenido en ácidos grasos libres y de productos secundarios de oxidación. La actividad lipoxigenasa fue menor ($p < 0.05$) al mes 1 en muestras tratadas; esta actividad disminuida no tuvo consecuencia en los compuestos primarios de oxidación pero está de acuerdo con los resultados obtenidos en los productos secundarios de oxidación al comparar ambas clases de muestras. A pesar de estas pequeñas diferencias obtenidas de los análisis bioquímicos entre pescado tratado y no tratado, la evaluación sensorial condujo al mismo período de vida (5 meses), aunque algunos cambios en piel y apariencia de la carne pudieron ser producidas como consecuencia del tratamiento antioxidante. Este trabajo constituye el primer intento de aplicar extractos acuosos de plantas a peces pelágicos enteros como paso previo a su comercialización como producto congelado. Investigación adicional que facilite una difusión más rápida de los antioxidantes en el músculo del pescado y el empleo de mezclas sinérgicas de compuestos antioxidantes puede evitar la pérdida de calidad y aumentar el período de vida durante el almacenamiento congelado de estos alimentos.

PALABRAS-CLAVE: Caballa - Calidad - Congelación - Lino - Pescado entero - Rancidez.

SUMMARY

Rancidity inhibition study in frozen whole mackerel (*Scomber scombrus*) by a previous plant extract treatment.

The effect of flaxseeds (*Linum usitatissimum*) on rancidity development in frozen whole mackerel (*Scomber scombrus*) was studied. For it, fresh mackerel were dipped in flaxseeds aqueous

extract during 60 min, frozen at -80°C during 24 hours and kept frozen (-20°C) up to 12 months. Sampling was carried out on the initial material and at months 1, 3, 5, 7, 9 and 12 of frozen storage at -20°C. A parallel experiment with non treated fish was carried out in the same conditions. Rancidity development was measured by several biochemical indices (free fatty acids, peroxides, conjugated dienes and trienes, secondary oxidation products and lipoxigenase activity) and complemented by the sensory analysis (skin, flesh odour, consistency and flesh appearance). As a result of the previous antioxidant treatment, peroxides showed to breakdown faster ($p < 0.05$) after month 7, so that higher ($p < 0.05$) contents on conjugated dienes and trienes could be detected in treated fish. The antioxidant treatment also led to some lower free fatty acid and secondary oxidation compounds formation. Lipoxigenase activity was lower ($p < 0.05$) at month 1 in treated samples; this decreased activity had not consequences on primary lipid oxidation compound but agreed to results obtained on secondary oxidation when comparing both kinds of samples. In spite of these small differences obtained from the biochemical analyses between treated and untreated fish, the sensory assessment led to the same shelf-life time (5 months), although some performances on skin and flesh appearance could be outlined as a result of the antioxidant treatment. The present work provides a first attempt for applying an aqueous plant extract to a pelagic whole fish as a previous step to its commercialisation as a frozen product. Further research enabling a faster diffusion of antioxidant compounds to the fish muscle and employing synergic mixtures of antioxidant compounds is encountered to avoid quality loss and attain a larger shelf-life time during the frozen storage.

KEY-WORDS: Flaxseeds - Frozen storage - Mackerel - Quality - Rancidity - Whole piece.

1. INTRODUCTION

Most marine species give rise to products of great economic importance in many countries. Fatty fish is attracting a great attention because of the positive role of marine lipids on human nutrition and health (Illingworth and Ullmann, 1990; Simopoulos, 1997). In this sense, there is an increasing interest in commercialising it in the frozen state (Erickson, 1997; Undeland and Lingnert, 1999), although its shelf life is known to be relatively short because of enzymatic and non enzymatic (Mohri et al., 1992; Richards and Hultin, 2002) rancidity development of the highly unsaturated lipid composition (Kolakowska, 2003).

To extend lag phase as long as possible and accordingly, retard lipid oxidation, a great attention is being given to the employment of natural antioxidants

(Frankel, 1995; Decker, 1998). Recent efforts are focused on the positive role of antioxidant molecules present in plant extracts (Yanishlieva and Marinova, 1996; Miyake and Shibamoto, 1997). One of such plant products is flaxseed (*Linum usitatissimum*, L.), composed from high ω 3 fatty acid (α -linolenic, specially) and phenolic compound (lignans; >500 μ g/g) contents (Oomah and Mazza, 2000). Flaxseed has been reported to be an important functional food because of its cancer prevention activity (Caragay, 1992), cardiovascular disease reduction (Ferreti and Flanagan, 1996) and pro-inflammatory mediator inhibition (James et al., 2000).

One abundant pelagic fatty fish species in both North Atlantic coasts is Atlantic mackerel (*Scomber scombrus*) belonging to the *Scombridae* family (FAO, 2004). Although it is recognised as a healthy food because of being a good source of high quality nutrients, particularly ω 3 fatty acids (Hardy and Keay, 1972; Leu et al., 1981), remains underutilised basically because of its poor frozen shelf life. Thus, previous research has shown an important endogenous prooxidant activity (Decker and Hultin, 1990; Saeed and Howell, 2001) and quality loss during the frozen storage (Jiang et al., 1987; Jia et al., 1996) and further processing (Zotos et al., 1995).

Successful applications of plant extract treatments have been carried out on frozen minced fish (Ramanathan and Das, 1992; Boyd et al., 1993) and fish fillets (Vareltzis et al., 1997; Saeed and Howell, 2002). However, research focused on whole fish species is scarce and to our knowledge, based on maintaining the colour stability of rockfish species, such as *Sebastes alascanus* (Wasson et al., 1991), *Sebastes ruberrimus* and *Sebastes alutus* (Li et al., 1998). The present work concerns frozen Atlantic mackerel trading as a whole fish product. On it, fish is treated with an aqueous extract of flaxseeds to enlarge its lipid stability during the frozen (-20°C) storage and accordingly, its quality and consumer acceptance. Lipid damage is monitored up to 12 months of storage by sensory and biochemical analysis.

2. EXPERIMENTAL PART

2.1. Flaxseeds extract preparation

A mixture consisting of 40.5g flaxseeds and 15 litres water was stirred during 15 min in an isothermal room (4°C). Then, it was centrifuged (3000g; 4°C) and the supernatant was employed immediately for fish treatment.

2.2. Raw fish, sampling and processing

Fresh mackerel (*Scomber scombrus*) were captured in September 2001 and kept on ice till arrival to the

laboratory (8 hours). All fish collected for the present study were males. Their gonads were at 5th /6th stage of the Maier's scale of gonad maturity.

Part of the fish was directly packaged in polyethylene bags and immediately frozen at -80°C. The other group was dipped in the above mentioned aqueous extract of flaxseeds (0.75 g flaxseeds / 100 g fish) in an isothermal room at 4°C. After 60 min, the fish were removed, packaged in polyethylene bags and frozen at -80°C. After 24 hours at -80°C, all fish were placed at -20°C. Sampling was undertaken at 1, 3, 5, 7, 9 and 12 months of frozen storage at -20°C and on the starting material. Once the fish were analysed by sensory assessment, the white muscle was separated, minced and employed for biochemical analysis. For both untreated and treated samples, three different fish batches were considered and studied separately to achieve the statistical study.

2.3. Sensory analysis

Sensory analysis was conducted by a taste panel consisting of five experienced judges, according to the guidelines presented in Table I (Council Regulation, 1990). Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and rejectable quality (C). Sensory assessment of the whole fish samples included the following parameters: skin, flesh odour, consistency and flesh appearance.

2.4. Water and lipid analyses

Water content was determined by weight difference between the homogenised fish muscle (1-2 g) and after 24 hr at 105 °C. Results were calculated as g water/100 g muscle.

Lipids were extracted by a chloroform-methanol mixture (2:1) according to Linko (1967). Quantification results are expressed as g total lipids/100 g wet muscle.

2.5. Lipid damage measurements

Free fatty acids (FFA) content was determined by the Lowry and Tinsley (1976) method based on complex formation with cupric acetate-pyridine. Results are expressed as g FFA/100 g lipids.

The content of lipid hydroperoxides and secondary oxidation compounds was determined according to the method of Schmedes and Hølmer (1984) and expressed in both cases as mg malondialdehyde/kg muscle.

Conjugated dienes (CD) and trienes (CT) content was determined (232 nm and 267 nm, respectively) in chloroform layer extracts (Paquot, 1979) and expressed as absorption coefficient (AC), according to the formula: $AC = A / w \times b$, where A is the

Table I
Scale employed for evaluating quality of frozen mackerel

Attribute	E (Highest quality)	A (Good quality)	B (Fair quality)	C (Rejectable quality)
Skin	Very intense pigmentation; transparent mucus	Insignificant pigmentation losses; slightly turbid mucus	Pigmentation discoloured and without shine; milky mucus	Important pigmentation losses; opaque mucus
Flesh Odour	Sharp seaweed and shellfish	Weak seaweed and shellfish	Slightly sour and incipient rancidity	Sharply sour and rancid
Consistency	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors
Flesh Appearance	Strongly hydrated and pink; myotomes totally adhered	Still hydrated and pink; myotomes adhered	Slightly dry and pale; myotomes adhered in groups	Yellowish and dry; myotomes totally separated

absorbance reading, w denotes the lipid mass (g) per 100 ml of the final solution used for measurement and b is the cell length (cm).

2.6. Lipoyxygenase activity

Lipoyxygenase was extracted with 10.0 mM phosphate buffer (pH 7.0), according to the Harris and Tall (1994) method modified by Stodolnik and Samson (2000). The activity of lipoyxygenase was studied through incubation according to the Slabyj and Hultin (1984) method with some modifications (Stodolnik and Samson, 2000). The enzyme activity was expressed according to the malondialdehyde content (nmol malondialdehyde/mg protein) produced after 24 h incubation of the mixture.

2.7. Statistical analyses

Biochemical data were subjected to the one-way ANOVA analysis ($p < 0.05$) (Statsoft, 1994); comparison of means was performed using a least-squares difference (LSD) method. Correlation analysis with time of the different parameters was studied (Statsoft, 1994); for sensory values, the Spearman test was employed.

3. RESULTS AND DISCUSSION

3.1. Water and lipid contents

Water content in the mackerel white muscle ranged between 67.1% and 71.3%; lipid content

ranged between 8.54% and 14.33%. In the present case, mackerel was captured in the period of the highest lipid content (Hardy and Keay, 1972; Leu et al., 1981). Variations in both constituents (water and lipids) contents may be explained as a result of individual fish variation, and not arising from frozen storage or antioxidant treatment. Comparison of the actual results with previous research showed a lower water content than leaner fish species (blue whiting, hake and cod) (Aubourg, 1999; Aubourg and Medina, 1999) in accordance with a common inverse ratio between water and lipid matter (Piclet, 1987).

3.2. Lipid hydrolysis

As being a fatty fish species, the FFA content (Figure 1) of the initial material was fast lower than in the case of medium fat (horse mackerel) (Aubourg, 2001a) and lean (blue whiting, cod, haddock) (Aubourg, 1999; Aubourg and Medina, 1999) fish species. Actual mean values showed a gradual increase in both kinds of samples with storage time. A very good agreement of FFA content increase with storage time was observed for untreated and treated samples ($r^2 = 0.96$ and $r^2 = 0.98$, respectively). The flaxseeds treatment led to lower mean values along the whole storage; however, differences were only significant ($p < 0.05$) at months 1 and 12.

Examining the extent of lipid hydrolysis was considered important to the study because of the important lipid hydrolysis development previously obtained in mackerel during frozen storage (Hwang and Regenstein, 1993; Zotos et al., 1995) and also

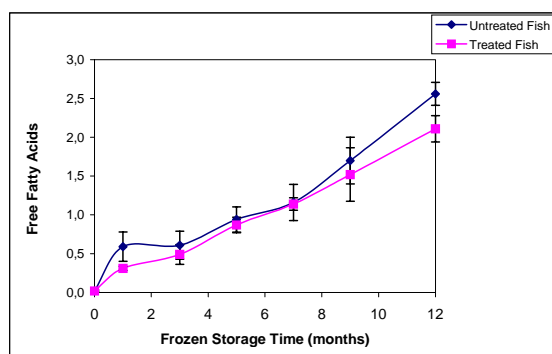


Figure 1

Free fatty acid assessment (g /100g lipid) during frozen storage of untreated and treated whole mackerel*
 * Bars denote standard deviation of the means.

because of the great incidence of free fatty acids on lipid oxidation (Miyashita and Takagi, 1986; Aubourg, 2001b).

3.3. Lipid oxidation

Hydroperoxides formation occurred during storage in both untreated and treated samples and reached the same top values at month 7 (Figure 2). After that, a faster ($p < 0.05$) hydroperoxide breakdown could be observed as a result of flaxseed presence till the end of the experiment.

Conjugate dienes (Figure 3) and trienes (Figure 4) were produced in parallel during the first seven months for both untreated and treated samples. Then, higher ($p < 0.05$) values were obtained in treated samples, presumably as a consequence of the higher decomposition of hydroperoxides (Figure 2). Conjugated diene fatty acids have demonstrated anticarcinogenic properties (Chin et al., 1992; O'Shea et al., 1998) and therefore the usage of aqueous flax extracts for this kind of fish material during its frozen storage would be beneficial.

Formation of secondary oxidation products followed the same trend for both treated and untreated samples (Figure 5). In both cases, a continuous increase led to the top value at month 5 that was followed by a sharp decrease ($p < 0.05$). Comparison between both kinds of samples showed lower mean values for treated fish, although an inhibitory effect of flaxseeds ($p < 0.05$) was only detected at months 1, 7 and 9.

3.4. Lipoyxygenase activity

Both kinds of samples provided an increase ($p < 0.05$) in lipoyxygenase activity at month 1, that was followed by a gradual decrease till month 7, when negligible activity values were detected (Figure 6). Flaxseeds effect only provided activity differences at

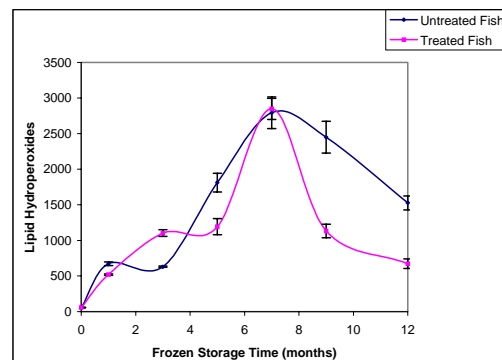


Figure 2

Lipid hydroperoxides determination (mg malondialdehyde/kg tissue) during frozen storage of untreated and treated whole mackerel*
 * Bars denote standard deviation of the means.

month 1, so that a greater ($p < 0.05$) lipoyxygenase activity was observed for control samples. This decreased activity had not consequences in primary lipid oxidation (Figure 2) but agreed to results obtained on secondary oxidation compounds formation (Figure 5).

An increase of lipoyxygenase activity in the first month of frozen storage was already observed (Samson and Stodolnik, 2001) for Baltic herring muscle when stored at -25°C . This increase was explained as a greater lipoyxygenase availability from cell structures after frozen storage release from cell membranes.

Previous research has already shown the presence in mackerel muscle of prooxidant compounds that may catalyse lipid oxidation (Harris and Tall, 1994; Jia et al., 1996). Indeed, Saeed and Howell (2001) showed that 12-LOX was the main promoter of the oxidation of mackerel lipids and could be partially inhibited by synthetic and natural antioxidants, although was active even at -70°C .

3.5. Sensory assessment

Progressive score decreases were observed with time for the four attributes considered (Table II), so that good correlation values ($r^2 = 0.94-0.96$) were obtained with the storage time for all the parameters in both kinds of samples. According to sensory acceptance scores, mackerel samples showed in both cases a shelf-life time of 5 months. However, treated samples provided a better quality score on flesh appearance at month 3 and skin attribute led to a longer acceptance time. In the case of untreated samples, the limiting factors were skin, flesh odour and flesh appearance, while flesh odour and flesh appearance were found the limiting factors for treated fish. When compared to biochemical indices, the best correlation values were obtained with the FFA formation ($r^2 = 0.81-0.93$).

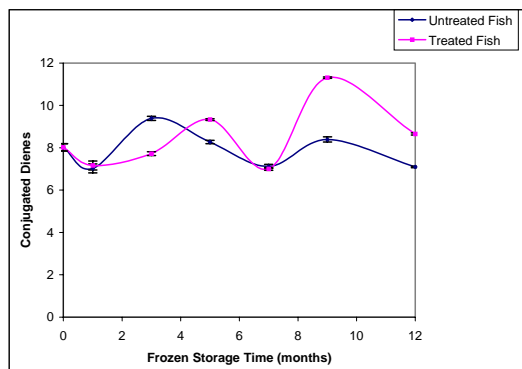


Figure 3
Conjugated dienes assessment (absorption coefficient) during frozen storage of untreated and treated whole mackerel*
* Bars denote standard deviation of the means.

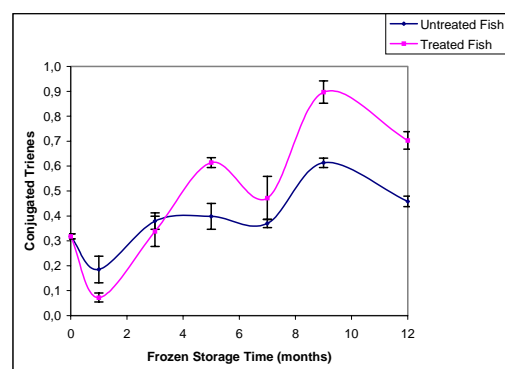


Figure 4
Conjugated trienes determination (absorption coefficient) during frozen storage of untreated and treated whole mackerel*
* Bars denote standard deviation of the means.

Table II
Sensory acceptance* of untreated (UT) and treated (T) frozen mackerel

Frozen Storage Time (months)	Skin		Flesh Odour		Consistency		Flesh Appearance	
	UT	T	UT	T	UT	T	UT	T
1	A	A	A	A	A	A	A	A
3	B	B	B	B	A	A	B	A
5	B	B	B	B	A	A	B	B
7	C	B	C	C	B	B	C	C
9	C	C	C	C	C	C	C	C
12	C	C	C	C	C	C	C	C

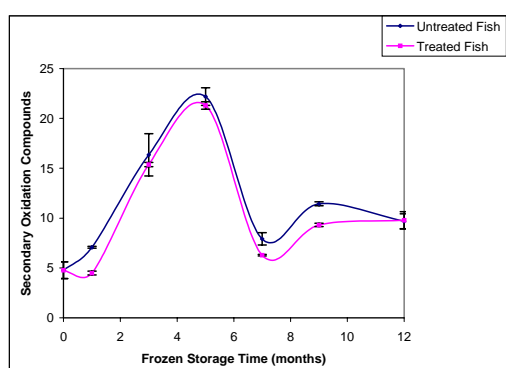


Figure 5
Secondary oxidation compounds determination (mg malondialdehyde/kg tissue) during frozen storage of untreated and treated whole mackerel*
* Bars denote standard deviation of the means.

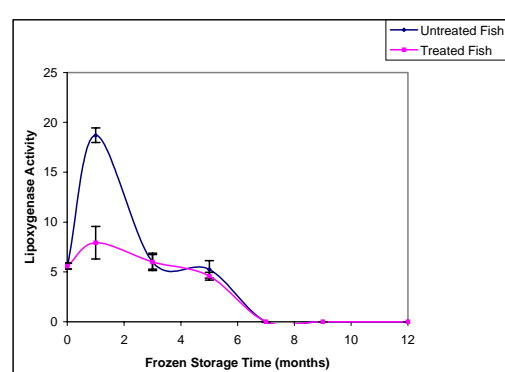


Figure 6
Lipoxigenase activity assessment (nmol malondialdehyde/mg protein) during frozen storage of untreated and treated whole mackerel*
* Bars denote standard deviation of the means.

4. FINAL REMARKS

In the present research, lipid hydrolysis and oxidation of frozen mackerel fish presented as whole

pieces were studied by biochemical analyses that were complemented by the sensory assessment. As a result of the previous antioxidant treatment of frozen storage, small advantages could be found.

Thus, a faster breakdown of hydroperoxides was observed after month 7, that led to a higher retention of conjugated dienes and trienes in treated fish. The plant extract also produced some lower lipid hydrolysis and secondary oxidation compound formation. In spite of these small differences, the same shelf-life time (5 months) was obtained for both kinds of samples, although some performances on skin and flesh appearance could be outlined as a result of the antioxidant treatment.

Among the different biochemical indices checked, FFA has shown to be the most reliable to assess the quality loss in both treated and untreated fish, since a good correlation value was obtained with time and with sensory marks. FFA formation has been reported to be strongly interrelated to lack of acceptability (Refsgaard et al., 2000).

Bibliography accounts for a large number of experiments where frozen mackerel prepared as minced (Kelleher et al., 1992; Hwang and Regenstein, 1995) and fillet (Chapman et al., 1993; Richards et al., 1998) products are successfully treated with natural antioxidants. The present work provides a first attempt for applying a plant extract treatment to a pelagic whole fish, which would be the most desirable way for traders to commercialise such kind of species in the frozen state. Further research enabling a faster diffusion of antioxidant compounds to the fish muscle should be encountered, and also research where different kinds of antioxidant compounds would be included, so that synergic effects could lead to a greater preservative behaviour and partially inhibit the quality loss.

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